Dr. Kalyan Giri

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Date of Birth: 14.06.1975 **Sex:** Male **Nationality:** Indian

Educational Background:

- **Ph.D.** from Jadavpur University, Kolkata (2006). Title of Thesis: "**Study of Protein Folding and Misfolding in relation to Disease**".
- **Post-M.Sc.** Diploma in ***Biophysical Sciences*** from Saha Institute of Nuclear Physics, Kolkata (2000).
- **M.Sc.** in ***Physics*** with ***Computer applications in Physics*** as special paper from Jadavpur University (1999); 1st Class.
- **B.Sc.** in ***Physics (Honours)*** with Chemistry & Mathematics as pass subjects from University of Burdwan (1996); 1st Class.

Research experience

I have been pursuing research in the Chemical Sciences Division of Saha Institute of Nuclear Physics, Kolkata, India since 2000. Presently I am working as Senior Research Fellow and my area of work is study of protein folding, misfolding and aggregation.

Outline of Research work:

My Ph.D. research work has revolved around the topic of protein folding. In the cell, protein folding is a highly controlled process with a cascade of proteins involved. Many proteins are, however, able to fold *in vitro* in absence of all these factors. This allows folding to be investigated by physical and chemical methods to probe the structural transition induced by environmental conditions (such as pH, temperature etc.) or the presence of denaturants. Information gathered from such experiments offers insight into the structure and stability of equilibrium intermediates on the folding pathway, which is crucial for a mechanistic understanding of the folding process and treatment of a wide range of diseases that can be linked to the aggregation of partially denatured or misfolded forms of proteins.

Studies of unfolding of proteins *in vitro* allow fundamental aspects of the mechanisms of folding to be uncovered without the complications of the biological environments. Our studies have dwelt on the unfolding of bovine prothrombin in the denaturants urea and GdmCl using biophysical methods. The fact that the transition curves obtained using different spectroscopic probes diverge indicates that a partially unfolded intermediate state occurs on the denaturant-induced unfolding pathway of prothrombin. These studies may also provide inputs to the development of theoretical approaches to protein folding. *In vitro* studies of the effect of pH changes on the structure of bovine prothrombin were performed by monitoring steady-state and time-resolved fluorescence properties of its intrinsic tryptophan residues and fluorescence resonance energy transfer between intrinsic (tryptophans) and extrinsic (ANS) fluorophores. The results show that at low pH there are structural changes in the protein that suggest formation of a non-native intermediate state.

Many protein deposition diseases are caused by mutations in essential cellular proteins leading to misfolding and aggregation of the proteins. Understanding the mechanisms of these diseases and their subsequent treatment require an understanding of how the relevant proteins fold or misfold. Our study presents a system of model peptides (containing 7, 11 and 17 successive alanines) mimicking the N-terminal sequence of the protein, PABP2 with a view to explore the effect of alanine repeat number on the pathological properties of peptides related to the disease OPMD. It was found that a peptide containing 11 successive alanine residues shows concentration-dependent intermolecular association into β–sheets and is highly effective in inducing apoptosis, a trait not exhibited by a similar peptide containing a 7-alanine stretch that consistently adopts α–helical conformation over the same range of concentrations. Aggregation-prone polyalanine peptides (11-ala and 17-ala) were used to study the kinetics of their fibril formation and its dependence on solution conditions (e.g., pH) and alanine repeat length. The fibrils formed in solution by 11-ala and 17-ala were found to be capable of forming higher order organized structures when exposed to suitable conditions. Peptide solutions, after incubation at high pH (>10) for appropriately long periods, when deposited on a glass slide and allowed to dry in air formed highly ordered, fractal-shaped aggregates of fibrils. 7-ala did not show any evidence of formation of fibrils or patterns. Kinetic studies showed that both fibril formation and growth of fractal patterns are faster for 17-ala than for 11-ala, indicating a correlation between

longer alanine stretches and early onset of the disease. The hierarchy of structures formed by self-assembling peptides on substrates will prompt future work on design of novel materials based on directed assembly of the peptides.

Water/alcohol mixtures are hydrogen-bonding solvents that have been suggested as model solvents for studying the effects of membrane fields on the structure of peptides and proteins. Using circular dichroism spectroscopy (CD) and transmission electron microscopy we have studied the structural tendencies and aggregation propensities of the polyalanine peptides in solvent mixtures of water with simple (methanol, ethanol) and fluorinated (TFE and HFiP) alcohols. The relative contributions of different secondary structures in the measured spectra were enumerated by using the Singular Value Decomposition (SVD) procedure. Our studies showed that although all alcohols induce formation of secondary structure in the peptides, the aggregation and fibrillation tendencies of the peptides are markedly enhanced only in those solvents that induce formation of β–sheets but not α-helices. High concentration of the alcohols induced both 11-Ala and 17-ala (but not 7-ala) to self-associate to form amorphous aggregates and, after sufficiently long incubation periods, well-defined fibrillar structures. This shows that the β–sheet structure induced by the alcohols in 11-ala are crucial for initiating the polymerization process that leads to the formation of aggregates and fibrils. It is very likely that these altered structures allow the peptides to self-associate into large oligomers, which then proceed to form the critical nucleus on the pathway of formation of large-scale polymers or fibrils.

Experience in different techniques:

- Optical spectroscopy (absorption, fluorescence and circular dichroism spectroscopy) and time correlated single photon counting method.
- Different types of chromatographic methods including preparative HPLC
- Kinetic studies using the Stopped-flow method (on the Biologic SFM3)
- Protein purification, estimation and characterization
- Agarose and poly-acrylamide gel electrophoresis and Western blotting technique.
- Peptide synthesis
- Mass spectrometry
- Optical microscopy
- Transmission Electron Microscopy (TEM)
- Scanning Electron Microscopy (SEM)
- Atomic Force Microscopy (AFM)
- Cell Culture and Detection of apoptosis

Computer programming language known: FORTRAN.

Membership of Learned Societies: Life member of Indian Biophysical Society (IBS).

Publications:

1. Kalyan Giri, Utpal Ghosh, Nitai P. Bhattacharyya and Soumen Basak, "Caspase 8 mediated apoptotic cell death induced by β-sheet forming polyalanine peptides", *FEBS Letters* **555 (**2003) 380-384.

2. Kalyan Giri, Nitai P. Bhattacharyya and Soumen Basak, "pH-dependent self-assembly of polyalanine peptides" *Biophysical Journal* **92** (2007) 293-302.

3. Kalyan Giri and Soumen Basak, "Conformation induction and aggregation in polyalanine peptides by organic solvents" (manuscript under preparation).

4. Kalyan Giri and Soumen Basak, "Urea and GdmCl induced unfolding of bovine Prothrombin" (manuscript under preparation).

5. Kalyan Giri and Soumen Basak, "Fluorescence resonance energy transfer as a spectroscopic probe to study pH-dependent structural variation of bovine prothrombin" (manuscript under preparation).

Presentations at Conferences/Symposia:

1. "Chemical denaturation studies of intact prothrombin", K. Giri, D. Debnath, K. Mukhopadhyay, A. Chakrabarti and S. Basak, *National Symposium on Biophysics (organized by Indian Biophysical Society)*, Indian Institute of Chemical Biology, Kolkata, 15-17 January 2001.

2. "Solvent-dependent aggregation behaviour of polyalanine peptides", K. Giri, N. P. Bhattacharya and S. Basak, *Discussion Meeting on Structural Biology and Symposium on Biophysics (organized by Indian Biophysical Society)*, Madras University, Guindy Campus, Chennai, 21-23 January 2002.

3. "Protein aggregation and disease: a conformational study of model peptides", K. Giri, N. Bhattacharya and S. Basak, *National Conference on Recent Trends in Biology Inspired Physics*, S. N. Bose National Centre for Basic Sciences, Kolkata, 18-21 March 2002.

4. "*In vitro* peptide aggregation enhances cell death", K. Giri, U. Ghosh, N. P. Bhattacharya and S. Basak, *First Indian Symposium of the Protein Society*, Indian Institute of Technology (Bombay), Mumbai, 18-20 October 2002.

5. "Correlation of aggregation and cellular toxicity with increasing alanine repeats in polyalanine peptides", K. Giri, N. P. Bhattacharya and S. Basak, *71st*

Annual Meeting of Society of Biological Chemists (India), Punjab Agricultural University, Ludhiana, 14-16 November 2002.

6. "Apoptosis induction by conformation-specific polyalanine peptides", K. Giri and S. Basak, *NCBS Symposium on Molecules, Machines and Networks*, National Centre for Biological Sciences, Bangalore, 5-9 January 2004.

7. "Fractal-like growth patterns of aggregating polyalanine peptides", K. Giri, N. P. Bhattacharyya and S. Basak, *Symposium on Pattern Formation in Nonequilibrium Systems (Satellite Meeting of STATPHYS 2004)*, S. N. Bose National Centre for Basic Sciences, Kolkata, 11-13 July 2004.

8. "Aggregation and pattern formation by polyalanine peptides at high pH", K. Giri, N. P. Bhattacharyya and S. Basak, *National Symposium on Recent Trends in Molecular & Medical Biophysics*, University of Pune, 22-25 January 2005.

9. "Nucleation-controlled polymerization of polyalanine peptides in oculopharyngeal muscular dystrophy", K. Giri, N. P. Bhattacharyya and S. Basak, *National Symposium on Molecular Mechanism of Diseases and Drug Action*, Saha Institute of Nuclear Physics, Kolkata, 16-18 November 2005.

10. "Conformational properties of polyalanine peptides in alcohol-water mixtures: Circular Dichroism study and Singular Value Decomposition analysis", K. Giri and S. Basak, *National Symposium on Molecules, Interaction and Design: a Biological Perspective (IBS 2006)*, Saha Institute of Nuclear Physics, Kolkata, 7-9 January 2006.

References:

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